

IN THE CLAIMS

The status of each claim is provided below.

Claims 1-108: (Canceled).

109. (Currently Amended) A method of determining the initial amounts of individual species of a target gene, comprising:

amplifying a target gene and monitoring the amplification by real-time PCR, wherein the real-time PCR is accomplished with a nucleic acid probe which is capable of hybridizing to the target gene;

performing a polymorphous analysis selected from the group consisting of T-RELP (terminal restriction fragment length polymorphism), RFLP (restriction fragment length polymorphism), SSCP (single strand conformation) or CFLP (cleavage fragment length polymorphism) with respect to the amplified target gene to determine a polymorphous composition ratio of individual species of the target gene; and

determining the initial amount of the target gene from the percentage of a change in the intensity of fluorescence occurring as a result of hybridization the nucleic acid probe and the amplified target gene; and

determining the initial amounts of individual species of the target gene by multiplying the initial amount of the target gene by said polymorphous composition ratio of individual species of the target gene.

110. (Currently Amended) A method of determining the initial amounts of individual species of a target gene, comprising:

amplifying a target gene and monitoring the amplification by real-time PCR;

performing a polymorphous analysis with respect to the amplified target gene to determine a polymorphous composition ratio of individual species of the target gene; and

determining the initial amount of the target gene from the percentage of a change in the intensity of fluorescence occurring as a result of hybridization the nucleic acid probe and the amplified target gene; and

determining the initial amounts of individual species of the target gene by multiplying the initial amount of the target gene by said polymorphous composition ratio of individual species of the target gene,

wherein the real-time PCR is accomplished with a nucleic acid probe,

wherein the probe comprises a single-stranded oligonucleotide capable of hybridizing to the target nucleic gene,

wherein the probe is labeled with a fluorescent dye and a quencher substance,

wherein the oligonucleotide is labeled with the fluorescent dye and the quencher substance such that the intensity of fluorescence in a hybridization reaction system increases when the probe is hybridized with the target gene, and

wherein the oligonucleotide forms no stem-loop structure between bases at positions where the oligonucleotide is labeled with the fluorescent dye and the quencher substance.

111. (Previously Presented) The method of Claim 109, wherein the real-time PCR is accomplished with a nucleic acid probe,

wherein the probe comprises a single-stranded oligonucleotide capable of hybridizing to the target nucleic gene,

wherein the probe is labeled with a fluorescent dye and a quencher substance,

wherein the oligonucleotide is labeled with the fluorescent dye and the quencher substance such that the intensity of fluorescence in a hybridization reaction system increases when the probe is hybridized with the target gene, and

wherein the oligonucleotide forms no stem-loop structure between bases at positions where the oligonucleotide is labeled with the fluorescent dye and the quencher substance.

112. (Previously Presented) The method of Claim 109, wherein the real-time PCR is accomplished with a nucleic acid probe,

wherein the probe is labeled at an end portion thereof with a fluorescent dye, and the probe has a base sequence designed such that, when the probe hybridizes at the end portion thereof to the target nucleic acid, at least one G (guanine) base exists in a base sequence of the target gene at a position 1 to 3 bases apart from an end base of the target nucleic acid hybridized with the probe;

whereby the fluorescent dye is reduced in fluorescence emission when the probe labeled with the fluorescent dye hybridizes to the target gene.

113. (Previously Presented) The method of Claim 109, wherein the real-time PCR is accomplished with a nucleic acid probe,

wherein the probe is labeled at an end portion thereof with a fluorescent dye, and

wherein the probe has a base sequence designed such that, when the probe hybridizes to the target gene, plural base pairs in a probe-nucleic acid hybrid complex form at least one G (guanine) and C (cytosine) pair at the end portion;

whereby the fluorescent dye is reduced in fluorescence emission when the probe labeled with the fluorescent dye hybridizes to the target gene.

114. (Previously Presented) The method of Claim 109, wherein the real-time PCR is accomplished with a nucleic acid probe,

wherein the probe is labeled with a fluorescent dye, wherein the probe is labeled at a position other than a 5' end phosphate group or a 3' end OH group thereof with the fluorescent dye, and

wherein the probe has a base sequence designed such that, when the probe hybridizes to the target gene, plural base pairs in a probe-nucleic acid hybrid complex form at least one G (guanine) and C (cytosine) pair at the modification portion; whereby the fluorescent dye is reduced in fluorescence emission when the probe labeled with the fluorescent dye hybridizes to the target gene.

115. (Previously Presented) The method of Claim 110, wherein the polymorphous analysis is T-RELP (terminal restriction fragment length polymorphism), RFLP (restriction fragment length polymorphism), SSCP (single strand conformation) or CFLP (cleavage fragment length polymorphism).

116. (Previously Presented) The method of Claim 111, wherein the single-stranded oligonucleotide is labeled on the same nucleotide thereof with the fluorescent dye and the quencher substance.

117. (Previously Presented) The method of Claim 110, wherein the single-stranded oligonucleotide is labeled on the same nucleotide thereof with the fluorescent dye and the quencher substance.

118. (Previously Presented) The method of Claim 111, wherein the distance between the bases at the positions where the oligonucleotide is labeled with the fluorescent dye and quencher substance, respectively, is 1 to 20 bases.

119. (Previously Presented) The method of Claim 110, wherein the distance between the bases at the positions where the oligonucleotide is labeled with the fluorescent dye and quencher substance, respectively, is 1 to 20 bases.

120. (Previously Presented) The method of Claim 111, wherein the probe is labeled at a 3' end thereof with the fluorescent dye.

121. (Previously Presented) The method of Claim 110, wherein the probe is labeled at a 3' end thereof with the fluorescent dye.

122. (Previously Presented) The method of Claim 111, wherein the probe is labeled at a 5' end thereof with the fluorescent dye.

123. (Previously Presented) The method of Claim 110, wherein the probe is labeled at a 5' end thereof with the fluorescent dye.

124. (Previously Presented) The method of Claim 110, wherein the probe has G or C as a 5' end base and is labeled at the 5' end thereof with the fluorescent dye.

125. (Previously Presented) The method of Claim 110, wherein a hydroxyl group on a 3' carbon of ribose or deoxyribose at the 3' end or a hydroxyl group on a 3' or 2' carbon of ribose at the 3' end has been phosphorylated.

126. (Previously Presented) The method of Claim 111, wherein a hydroxyl group on a 3' carbon of ribose or deoxyribose at the 3' end or a hydroxyl group on a 3' or 2' carbon of ribose at the 3' end has been phosphorylated.

127. (Previously Presented) The method of Claim 111, wherein the probe is labeled at a 5' end phosphate group and/or a 3' end phosphate group thereof with the fluorescent dye.

128. (Previously Presented) The method of Claim 110, wherein the probe is labeled at a 5' end phosphate group and/or a 3' end phosphate group thereof with the fluorescent dye.

129. (Previously Presented) The method of Claim 111, wherein the oligonucleotide of the probe is a chemically-modified nucleic acid.

130. (Previously Presented) The method of Claim 110, wherein the oligonucleotide of the probe is a chemically-modified nucleic acid.

131. (Previously Presented) The method of Claim 129, wherein the chemically-modified nucleic acid is 2'-O-methyloligonucleotide, 2'-O-ethyloligonucleotide, 2'-O-butyloligonucleotide, 2'-O-ethyleneoligonucleotide, or 2'-O-benzyl-oligonucleotide.

132. (Previously Presented) The method of Claim 130, wherein the chemically-modified nucleic acid is 2'-O-methyloligonucleotide, 2'-O-ethyloligonucleotide, 2'-O-butyloligonucleotide, 2'-O-ethyleneoligonucleotide, or 2'-O-benzyl-oligonucleotide.

133. (Previously Presented) The method of Claim 110, wherein the oligonucleotide of the probe is a chimeric oligonucleotide which comprises a ribonucleotide and a deoxyribonucleotide.

134. (Previously Presented) The method of Claim 111, wherein the oligonucleotide of the probe is a chimeric oligonucleotide which comprises a ribonucleotide and a deoxyribonucleotide.

135. (Previously Presented) The method of Claim 133, wherein the chimeric oligonucleotide comprises 2'-O-methyloligonucleotide, 2'-ethyloligonucleotide, 2'-O-butyloligonucleotide 2'-O-ethyleneoligonucleotide, or 2'-O-benzyl-oligonucleotide.

136. (Previously Presented) The method of Claim 134, wherein the chimeric oligonucleotide comprises 2'-O-methyloligonucleotide, 2'-ethyloligonucleotide, 2'-O-butyloligonucleotide 2'-O-ethyleneoligonucleotide, or 2'-O-benzyl-oligonucleotide.

137. (Previously Presented) The method of Claim 109, wherein said polymorphous analysis is T-RELP.

138. (Currently Amended) The method of Claim 137, wherein said T-REFP is an analysis, wherein the amplified target gene, is digested by making use of at least one endonuclease selected from Bso FI, Hha I, Hph I, Mn1 I, Alu I and Msp I.

139. (Previously Presented) The method of Claim 138, wherein said restriction endonuclease is at least one endonuclease selected from Rca I, Alu I and Msp I.

140. (Previously Presented) The method of Claim 139, wherein said restriction endonuclease is Hha I.

141. (Currently Amended) The method of Claim 140, wherein the gene fragments obtained ~~obtaining~~ by said digestion are analyzed and determined using a sequencer or HPLC.

142. (Previously Presented) The method of Claim 137, wherein said quantitative real-time monitoring PCR method makes use of a nucleic acid probe labeled with a fluorescent dye at the 5'-end thereof, wherein the fluorescent dye is one dye selected from FITC, Texas red, 6-Joe, TMR, Alexa 488, Alexa 532, "BODIPY FL/C3", and "BODIPY FL/C6".

143. (Currently Amended) A method for determining the initial amounts of individual species of a target gene, comprising:

(1) amplifying a target gene and monitoring the amplification by a quantitative real-time PCR making use of a fluorescence-quenching probe labeled with a fluorescent dye at the 5'-end thereof as a primer(s);



- (2) digesting the amplified target gene using an endonuclease;
- (3) thermally modifying the obtained gene fragments ~~is thermally modified~~ into single-stranded forms
- (4) detecting gene fragment(s) labeled with a fluorescent dye at the 5'-end thereof by a sequencer or HPLC by measuring a fluorescent emission in the fluorescent dye as a signaling marker;
- (5) measuring the fluorescent intensity of fragment peaks detected by the sequencer or HPLC, which peaks are caused by the gene fragments, and then, determining a composition ratio of individual fragments; and
- (6) determining the initial amount of the target gene from the percentage of a change in the intensity of fluorescence occurring as a result of hybridization the nucleic acid probe and the amplified target gene; and
- (7) determining the initial amounts of individual species of the target gene by multiplying the initial amount of the target gene by said composition ratio of individual fragments,

wherein said fluorescence-quenching probe is labeled at a phosphate group or a 5'-OH group of a ribose or deoxyribose which is obtained by dephosphorization of the 5'-end, and has a base sequence designed such that, when the probe hybridizes at the end portion thereof to the target nucleic acid, at least one G (guanine) base exists in a base sequence of the target gene at a portion 1 to 3 bases apart from end base of the target gene hybridized with the probe.

144. (Previously Presented) The method of Claims 111 or 115, wherein said polymorphous analysis is T-RELP.

145. (Currently Amended) The method of Claim ~~144~~ 143, ~~wherein said T-REFP is an analysis~~, wherein the amplified target gene is digested by making use of at least one endonuclease selected from Bso FI, Hha I, Hph I, Mnl I, Rca I, Alu I and Msp I.

146. (Previously Presented) The method of Claim 144, wherein said restriction endonuclease is at least one endonuclease selected from Rca I, Alu I and Msp I.

147. (Previously Presented) The method of Claim 145, wherein said endonuclease is Hha I.

148. (Previously Presented) The method of Claim 143, wherein the gene fragments obtained by said digestion are analyzed and determined using a sequencer or HPLC.

149. (Previously Presented) The method of Claims 110 or 111, wherein said quantitative real-time monitoring PCR method makes use of a nucleic acid probe labeled with a fluorescent dye and a quencher, wherein the fluorescent dye is one dye selected from FITC, EDANS, Texas red, 6-Joe, TMR, Alexa 488, Alexa 532, "BODIPY FL/C3", and "BODIPY FL/C6"; the quencher is one dye selected from Dabcyl, "QSY7", "QSY33", Ferrocene, methyl viologen, and N, N'-dimethyl-2,9-diazapyrenium.

SUPPORT FOR THE AMENDMENTS

The amendments to the claims are supported by the specification, in particular at page 13, bottom; page 102, bottom; and Example 38. Accordingly, no new matter is believed to have been added to the present application by the amendments submitted above.